Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Overview of Triplet Repeat Disorders

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Slide 1:
Hello, my name Neal Z. Niu. I am an assistant professor at Baylor College of Medicine in Houston, Texas. Welcome to this Pearl of Laboratory Medicine on "Overview of Triplet Repeat Disorders."

Slide 2:
In this short session, we will go over some classic triplet repeat disorders together, discuss some interesting clinical features associated with the triple repeat expansion, and also highlight several points for consideration on molecular testing, such as Southern blotting and PCR-based assays.

Slide 3:
Triplet or trinucleotide repeat is a type of short tandem repeat in our genome. The sizes of these repeats are often polymorphic, with great variation in the general population. The most important feature for triplet repeat is that they are often not stable. Depending on what the sequence is, what the original repeat size is, and what genomic context it is located in, about 10-100% of triplet repeat elements can expand when passed to the next generation. When the size of the triplet repeat exceeds a disease threshold, which is specific for each gene and specific disorder, the expanded repeats will affect gene function, leading to specific clinical phenotype. More than 20 neurodegenerative and neuromuscular diseases are caused by the instability of triplet repeat expansion. Most well known triplet disorders exhibit autosomal dominant inheritance such as Huntington disease, or X-linked such as Fragile X syndrome. These two disorders will be discussed in more detail in other Pearls.

Slide 4:
We can simply group triplet repeat disorders based on whether the triplet repeat is located in the coding region of the gene. A group of nine disorders are caused by the expansion of CAG repeats in the coding region in nine different genes. This group includes Dentatorubral-pallidoluysian atrophy (DRPLA), Huntington, Kennedy, and several types of spinocerebellar ataxia. Because CAG encodes amino acid glutamine, CAG repeat will be translated into polyglutamine stretch in the protein. This group is also referred as polyglutamine or polyQ disorders.

Slide 5:
In this table, we can see that variable CAG repeat sizes are observed in the healthy individuals. Each disorder has its own threshold for triplet repeat size. For the polyglutamine disorders, pathogenic repeat size is normally within a hundred.

Slide 6:
Expansions of other triplet repeats in the coding region, such as those encode poly-alanine, have been recently studied in a group of congenital malformations, such as skeletal dysplasia and nervous system anomalies. Compared to polyglutamine disorders, pathogenic repeat expansions of poly-alanine are often relatively small. In this group of disorder, poly-alanine stretches are often identified in the transcription factors which are important for development.

Slide 7:
Another large group of classic triplet repeat disorders are due to repeat expansion in the non-coding region, such as the CGG repeat located in the 5' untranslated region of *FMR1* gene which causes fragile X syndrome, or the CTG expansion in the 3' untranslated region of *DMPK* gene which causes myotonic dystrophy type I. It is worth mentioning that the expansion of GAA repeats in the first intron of frataxin gene causes Friedreich ataxia, which is the only common triplet repeat disorder having an autosomal recessive inheritance pattern. Relatively large repeat expansions are observed in patients of this group of disorders: repeat size greater than one hundred are common.

Slide 8:
Also from this table, it is very clear that there is some distance between the repeat size considered normal to the repeat size considered pathogenic for each condition. This gap includes repeat expansion regarded as premutation and some interpretation gray zone.

Slide 9:
As we just mentioned, normal triple repeat size are observed in normal population. These are believed to be relatively stable with less change of expansion.

The premutation repeat size is greater than normal, but people carrying premutation do not have that disorder. However, their children will be at risk of inheriting the premutation allele which may expand into a fully penetrant pathogenic mutation.

There are also some interpretation gray zones for those triplet sizes falling between normal and premutation or between premutation and full mutation.

Slide 10:
For example, 35-49 CTG repeat in the 3'UTR region of the *DMPK* gene is considered premutation. Individuals carrying a premutation in *DMPK* are asymptomatic. But when their children inherit this allele, which expands to above 50 CTG repeat, it will lead to myotonic dystrophy.

Slide 11:
Another interesting feature of triplet repeat disorders is that the repeat size correlates well with the clinical phenotype of each disease.

Still using myotonic dystrophy as an example. Here is a three generation family affected with myotonic dystrophy. The grandmother of the proband has one allele of 100 CTG repeat in the *DMPK* gene. She presented with cataracts diagnosed at the age of 50. The mother of the proband inherited the disease allele, which expanded to about 500 CTG repeat and she was diagnosed with myotonia, facial weakness, --classic myotonic dystrophy phenotype at age of 40. The proband was diagnosed at birth with facial weakness, and severe neonatal hyotonia, and molecular testing detected a CTG repeat size greater than ~1500.

Clinically, based on the severity and age of onset, myotonic dystrophy is categorized to mild, classic, and severe congenital forms. Molecularly, the sizes of CTG expansion correlate very well with the clinical phenotype.

Slide 12:
This pedigree is also an example of a phenotype called "anticipation." In genetic disorders, anticipation refers to increased severity, increased disease penetrance in the subsequent generation, and decreased age of onset. Anticipation has been found in many genetic disorders with still unclear mechanisms, but for triplet repeat disorders, the repeat expansion through generation provides some molecular basis for these phenomena.

Slide 13
Also very interestingly, the likelihood of an expansion event is affected by whether it is inherited from the mother or the father. For example, the triplet repeat in myotonic dystrophy is most likely to expand when inherited from the mother. But the repeat for Huntington disease is most likely to expand when inherited from the father.

Slide 14:
The stretches of triplet repeat at the DNA level may form secondary structure, which is determined by the repeat sequence, the length of the repeat, and very importantly, the genomic context, such as whether the repeats are interrupted by other sequences.

Slide 15:
When these secondary structures are formed in the triplet repeats, they may affect DNA replication. Several models were proposed, such as the replication slippage model for repeat expansion shown here. The replication machinery may be paused due to secondary structures, and the slippage of the replication fork back or forward can give rise to an expansion or a contraction. This mechanism of repeat expansion is important to understand the high new mutation rate and the high frequency of somatic mosaicism, two features also important for the molecular diagnostic testing.

Slide 16:
Next, we will go over some examples of molecular testing methods for triplet repeat expansion disorders.

Slide 17:
Southern blot is a classic method to size triplet repeat especially for large expansion. Because no DNA amplification is involved, Southern blot has advantage in detecting mosaicism and homozygous alleles. Methylation status can be further examined with methylation sensitive restriction enzyme and this method is also relatively inexpensive.

The cons of Southern blot include limited resolution on small expansions and long turn around time.

Slide 18:
Here is an example of a Southern blot film for myotonic dystrophy. The lower band across all samples indicate the normal allele. In the sample pointed by the arrow head, a pathogenic allele with about ~2000 CTG repeat is detected. In the next sample pointed by the arrow, the expanded allele was detected with a diffused smear pattern between 1000 to 1400 repeats. This pattern suggested somatic mosaicism in this patient.

Slide 19:
Here is another Southern blot film for the detection of CGG expansion in the *FMR1* gene. In this test, methylation sensitive restriction enzyme was used to distinguish the methylated vs unmethylated alleles. Full mutation in the *FMR1* gene results in hypermethylation, and this abnormal methylation can be revealed with methylation sensitive enzyme. However, methylation in *FMR*I caused by the full mutation may not present in certain tissue type such as chorionic villus samples, and somatic mosaicism is common as indicated with the diffusive smear pattern.

Slide 20:
PCR-based assays offers many advantages such as accurate sizing of small triplet repeat and very fast turn around time. However, it should be noted that efficiency of PCR amplification is affected by the length of triplet repeat, and rare sequence polymorphisms do exist. When only one allele is detected by PCR-based assay, confirmation using alternative method is recommended. Amplification of triplet repeat with high GC content is sensitive to PCR condition requiring proper controls.

Slide 21:
PCR amplified product can be labeled with isotope in “Hot PCR” and measured in reference to molecular ladder. Similarly, fluorescence labeled primers have been commonly utilized in the molecular testing of many triplet repeat disorders, which can be finished in couple of days. The number of triplet repeat can be calculated based on the design such as two alleles, one with 17 repeats and the other with 35 repeat are shown in the right panel.

Slide 22:

How does triplet repeat affect the gene function? Pathogenic triplet repeat may result gene silence, such as full mutation in the Fragile X syndrome caused by abnormal methylation. Triplet expansion in the coding region can produce a protein with altered function. And interestingly, triplet expansion in myotonic dystrophy gives rise to toxic RNA which interferes with many other genes in the affected tissues. Hopefully, studies in the mechanisms of triplet repeat disorder will move the field beyond molecular diagnosis to therapy.

Slide 23: Summary

In summary, triplet repeat disorders are a group of genetic disorders caused by unstable triplet expansion. Because triplet repeat is not stable, these disorders often have high new mutation rate and somatic mosaicism. Each disorder has a threshold for triplet repeat size, greater expansion often results in severe phenotype. Premutation, anticipation, and parental effects are all interesting features of these disorders. Molecular genetic tests of triplet repeat disorder include Southern blot and PCR-based assays. And we have reviewed several examples of them.

Slide 24: References

Slide 25: Disclosures

Slide 26: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "Overview of Triplet Repeat Disorders." My name Neal Z. Niu.